A HIGHLY RESOLVED, OXYGEN-EVOLVING PHOTOSYSTEM II PREPARATION FROM SPINACH THYLAKOID MEMBRANES

EPR and electron-transport properties

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1. Introduction

Photosystem II of chloroplast thylakoid membranes provides light-generated oxidizing equivalents which ultimately oxidize water to oxygen. The system is known to contain a reaction center chlorophyll complex, P680, a quinone acceptor denoted as Q, a P680 donor molecule designated as Z and a high-potential cytochrome *b*-559 [1]. The relationship between these components and the presumed manganese protein thought to be the actual site of water-splitting is as yet poorly understood. Attempts to resolve this photoreaction from intact membranes of higher plant chloroplasts by detergent action have resulted in a variety of preparations ranging from reaction centers [2,3] to more complex assemblies with vesicular structure [3]. While these preparations are generally able to support electron transfer from an added donor to an exogenous acceptor, all higher plant preparations reported are unable to carry out oxygen evolution with high activity. Photosystem II may be isolated from membranes of the cyanobacterium *Phormidium laminosum* [4]. These preparations, in contrast to those from higher plants, retain high rates of oxygen evolution [4] and are enriched in EPR signal II with residual contamination from PS I, as shown by the presence of some signal I ($P700^+$) [5].

We have re-examined the methodology for the separation of PS II from spinach chloroplast thylakoid membranes; here we report the properties of oxygen-evolving PS II preparations obtained by detergent resolution of chloroplast thylakoid membranes.

2. Materials and methods

Highly active intact thylakoid membranes were prepared as in [6]. Subchloroplast membranes capable of oxygen evolution were prepared by suspension of the intact thylakoids (2 mg chl/ml) in $MgCl_2$ (5 mM), NaCl (15 mM) and Hepes buffer (20 mM, pH 7.5) and incubation with Triton X-100 (25 mg/mg chl) at 4°C for 30 min. The fraction of chl-containing material sedimenting at 40 000 × *g* (30 min) was resuspended in incubating buffer (2 mg chl/ml) with Triton X-100 (5 mg/mg chl), recentrifuged immediately (40 000 × *g*, 30 min) and stored in sucrose (0.4 M), $MgCl_2$ (5 mM), NaCl (15 mM) and Hepes (20 mM, pH 7.5) at −35°C for subsequent analyses. In this procedure, which is superficially similar to that in [3], we have produced a set of conditions with regard to salt concentrations whereby O_2 evolution activity is resistant to denaturation by exposure to Triton X-100. Procedures for assay of oxygen evolution, for Tris-inhibition, and EPR detection of signals II_s and II_f have been reported in [7,8]. Cytochrome content was assayed optically by using an Aminco DW-2 spectrophotometer.

3. Results

Fig.1 compares the EPR spectra of intact thylakoids and of PS II particles in the presence of ferricy-

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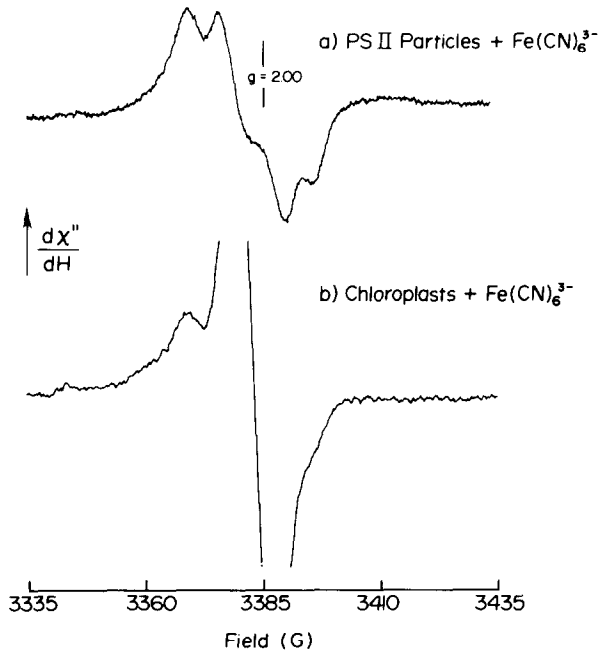

Fig.1. EPR spectra of PS II particles (A) and intact chloroplasts (B) in the presence of 10 mM ferricyanide. Instrument conditions: modulation amplitude, 4 Gpp; time constant, 200 ms; power, 20 mW; gains, 1 × 10^6 (PS II particles (5.4 mg chl/ml) and 1.6 × 10^6 intact chloroplasts (6.2 mg chl/ml); scan rate, 60 G/min; temp., 25°C.

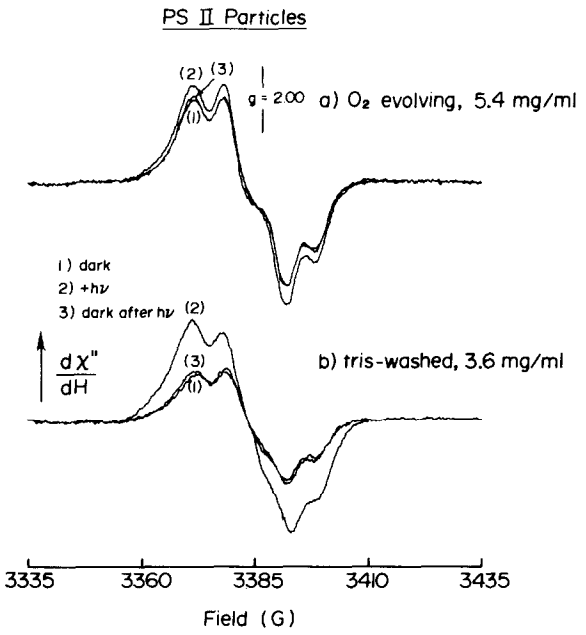

Fig.2. Effect of 0.8 M Tris (pH 8.0) on light-induced changes in EPR signal associated with PS II particles. The instrument gain was the same for both experiments; other instrumental conditions as given in fig.1; chlorophyll concentrations as indicated.

anide. In the absence of ferricyanide, the spectra of the 2 preparations are similar and show only the features of signal II_s, the stable PS II free radical [10] (not shown), whereas under strongly oxidizing conditions a substantial contribution from signal I ($P700^+$) is seen in intact thylakoid membranes which is absent from the PS II preparation. These data demonstrate that we are able to achieve essentially complete separation of PS I from PS II by the Triton treatment. Fig.2 shows the effect of illumination on changes in the EPR signal associated with the PS II preparation both before and after incubation of this material in 0.8 M Tris (pH 8.0). The doubling in signal II amplitude seen upon illumination of the inhibited preparation shows that signal II_f, which arises from the P680 donor Z, is induced by this treatment, a finding similar to that observed when oxygen evolution in intact thylakoid membranes is inhibited by Tris [9]. These EPR results suggest that the PS II preparation is capable of oxygen evolution; this supposition is confirmed by the data in table 1, where the rate of ferricyanide-supported oxygen evolution in the PS II particles is seen to be maximal at pH 6–7, whereas intact membranes produce optimal rates above pH 7. Table 1 demonstrates that the rate of oxygen evolution in the PS II particles is high: on a chlorophyll basis, the particle rate at pH 7 is comparable to that of intact thylakoids at pH 8; on a PS II unit basis, the particle rates are ⩾50% of the thylakoid rates (see below).

An inspection of fig.1–3a shows that the lineshape of signal II_s in the PS II preparation differs from that seen in intact, dark-adapted thylakoid membranes. This might be due to modification of PS II by the

Table 1
Effect of assay pH on oxygen-evolving activity of photosystem II membranes

pH	Activity[a] (μmol . h^{-1} . mg chl^{-1})	
	PS II membranes	Intact thylakoids
6.0	300	150
7.0	320	260
8.0	80	340

[a] Assayed with ferricyanide (3.5 mM) plus 2,5-dichloro-*p*-benzoquinone (250 μM)

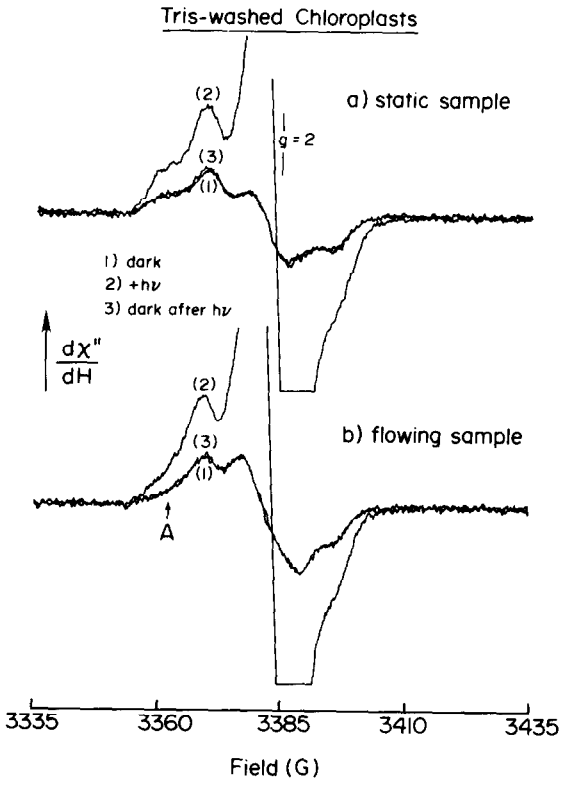

Fig.3. Effect of flow orientation on the lineshape of signal II_s and signal II_f in intact, Tris-washed chloroplast thylakoid membranes. Conditions as in fig.1, except that ferricyanide was absent; gain, 1×10^6; chl, 4 mg/ml.

action of Triton X-100, to signal I contamination, or alternatively, to an alteration in the orientation of the photosystem II membranes in the magnetic field in the EPR cavity. This possibility was tested in an experiment where Tris-treated thylakoid membranes were examined in an EPR flat cell under static and dynamic (flowing) conditions [11]. As shown in fig.3, the lineshape of signal II_s in flowing membranes is similar to that of II_s in the PS II preparation. Fig.2,3 also show that the lineshape of signal II_f reflects that of II_s regardless of the state of thylakoid or particle orientation. This is particularly clear in the low field region labeled 'A' in fig.3. Thus, we conclude that the apparent lineshape anomaly seen in the PS II preparation is due to an orientation phenomenon resulting from the applied magnetic field rather than to an artifact of Triton X-100 treatment.

Table 2
A comparison of photosystem II membranes and intact thylakoids

	Photosystem II	Intact thylakoids
Oxygen evolution	+	+
Photophosphorylation	–	+
Signal II_s	+	+
Signal II_f	+	+
Signal I ($P700^+$)	–	+
Cytochrome *b*-559	+	+
Cytochrome b_6	–	+
Cytochrome *f*	–	+

4. Discussion

Some properties of the PS II preparation described here are summarized briefly in table 2. We have not yet examined carefully the redox properties of cytochrome *b*-559 and its stoichiometry with regard to chl content of the PS II preparation. Of primary importance is the observation that active oxygen-evolving PS II preparations may be isolated from higher plant thylakoid membranes which, in contrast to the cyanobacterial preparations, are devoid of any PS I contamination. We show here for the first time the lineshape of signal II_f without signal I contamination, and show it to be: (a) identical to that of signal II_s; and (b) sensitive to orientation phenomena. Interestingly, it appears as if the orientational effects on the lineshapes of signal II_s and II_f are similar. This observation indicates that F^+ (the free radical responsible for signal II_s) [10] and Z^+ (the free radical giving rise to signal II_f) have similar orientations in the thylakoid membrane even though they appear to be functionally unrelated [9]. The signal II_{lt} species in [5,10] probably arises also from Z^+. The low temperatures employed in [5,10] prevent its rapid re-reduction by the oxygen-evolving complex following light generation while the oxidizing conditions eliminate cytochrome *b*-559 as a competing donor to the reaction center complex.

Although we cannot directly estimate the trap size of these photosystem II preparations by techniques available to us, we can provide an approximate stoichiometry based on Mn^{2+} content. Our work [13] on EPR-detectable Mn^{2+} in intact thylakoid membranes suggests that 4 Mn^{2+}/400 Chl are required for oxygen-evolving activity. Based on this number and the Mn^{2+} content of our PS II preparations, we estimate that

this material should contain about 1 trap/250 chl. The pH optimum for oxygen evolution in the PS II preparation ([6–7], table 1) is different from the optimum observed for this reaction in intact chloroplasts. A further examination of this phenomenon is now in progress to determine whether the effect reported here arises from pH effects on the oxygen-evolving reaction or from a pH-sensitive step on the reducing side of photosystem II.

Acknowledgements

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